INVESTIGATIONS OF THE CYTOCHROME C CYTOCHROME OXIDASE SYSTEM

BY

WALTER JOSEPH FRAJOLA

B.S., Hamline University, 1938
M.S., University of Illinois, 1947

AN ABSTRACT OF A THESIS
SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN
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INTRODUCTION

Keilin's spectroscopic studies of muscle pigments led to the rediscovery of the respiratory catalysts, which he named the cytochromes. They account for 80-90 per cent of the total respiration of many cells and tissues. The perfection by Warburg of a method for measuring the oxygen consumption of cells resulted in numerous investigations of the respiratory enzymes. Because cytochrome c has a characteristic absorption peak at 5500Å, spectrophotometric investigation of its respiratory activity is also possible. It was the purpose of this study to devise an improved method for the spectrophotometric assay of the cytochrome c-cytochrome oxidase system, to compare the method devised with the existing procedures, to study the kinetics of the system, to study the effect of heat treatment of cytochrome c on its biological activity, and to compare the spectrophotometric assay with the manometric method.

EXPERIMENTAL

Cytochrome c was prepared from frozen horse heart by the Keilin-Hartree procedure (1). Phosphate buffer homogenates of rat brain or rat liver served as the cytochrome oxidase. A suspension prepared by precipitating a phosphate homogenate with acetate and re-suspending in phosphate buffer according to the method of Wainio *et al.* (2) was also used as the oxidase. The spectrophotometric method employed was an improved modification of the procedure of Albaum, Tepperman and Bodansky (3). The measurements were carried out at wave length of 5495Å and a slit width of 0.025 mm. The cuvette contained 1.0 ml. of phosphate buffer of pH 7.4 and 1.9 ml. of a mixture of cytochrome c and water. Reduction was accomplished with a few grains of sodium hydrosulfite. After recording the optical density, moistened oxygen was bubbled through the solution for two minutes to remove excess hydrosulfite. After a three minute equilibration period without oxygen treatment, optical density measurements were begun. Several readings were taken at timed intervals before addition of the oxidase to determine the rate of auto-oxidation. Then 0.1 ml. of the oxidase was added rapidly with stirring and optical densities recorded at ten second intervals until the oxidation became negligible. An excess of sodium hydrosulfite was then added and the optical density

recorded. Compensation for the optical density due to the oxidase suspension was achieved by adding 0.1 ml. of the oxidase to a blank containing only the phosphate buffer.

The manometric experiments were conducted by the method of Schneider and Potter (4) as outlined by Maxwell (5).

RESULTS AND DISCUSSION

The rate of oxidation of reduced cytochrome c by cytochrome oxidase has been reported to be first order with respect to the cytochrome c. The experimental observations in the present studies are best explained by use of the equations of Elkins-Kaufman and Neurath (6). Neurath and Schwert (7) have recently reviewed the derivation and interpretation of these equations which express the rate of a reaction as the combination of a first and zero order reaction. With the aid of their interpretation, the rate of disappearance of reduced cytochrome c can be calculated. When this rate is plotted against initial concentration of reduced cytochrome c, the resulting curve is that of a rectangular hyperbola.

When the oxidase was kept constant and the substrate (cytochrome c) was altered by the heat treatment of Maxwell (5), comparison of the activity of the substrate was possible by comparison of the hyperbolic curves. The boiled cytochrome c was more active.

The cytochrome oxidase assay method of Schneider and Potter (4) depends upon a linear relationship between oxygen uptake and oxidase concentration at constant substrate concentration as proof that the oxidase is saturated with the substrate, cytochrome c. A manometric experiment was conducted in which this linearity was observed. When the minimal oxidase concentration was used in the spectrophotometric determination, it was observed that the cytochrome c was a limiting factor.

This led to attempts to reach a state of saturation of cytochrome oxidase with cytochrome c. Dilution of the oxidase resulted in a greatly diminished activity indicating the possibilities of alterations in the physical state of the enzyme or in the concentrations of cofactors or activating ions.

SUMMARY

1. A spectrophotometric method for the measurement of cytochrome oxidase activity has been described.

2. The cytochrome c-cytochrome oxidase reaction behaves kinetically as a combination first and zero order reaction.

3. Boiling cytochrome c solutions seems to enhance the biological activity of the residual cytochrome.

4. Comparison of the cytochrome oxidase activity of a preparation manometrically and spectrophotometrically indicates that the manometric results are low because the substrate concentrations are too low to saturate the enzyme.

5. Dilution of cytochrome oxidase suspensions in attempts to saturate the enzyme with substrate resulted in greatly diminished activity without achieving one-fourth saturation.

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VITA

The author was born in Chicago, Illinois, on November 2, 1916. He attended the elementary and secondary schools of Gilbert, Minnesota. His undergraduate training was received at Virginia Junior College, Virginia, Minnesota, and Hamline University, St. Paul, Minnesota. The Bachelor of Science degree with cum laude honors was received from the latter institution in June, 1938. From 1938 to 1942, he was employed as a teacher of chemistry, biology and physics in the public schools of Elk Point, South Dakota and Worthington, Minnesota. During the summers of that period he was enrolled in the graduate school of the University of Minnesota. From March, 1942, until induction into the Army of the United States in April, 1944, he was employed as an ammunition inspector by the Chicago Ordnance District. Upon completion of his army service in September, 1946, he entered the graduate school of the University of Illinois as a Teaching Assistant in Chemistry, a position he has held to this date. He received a Master of Science degree from the University of Illinois in October, 1947.

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